Original article

Thyroid Stimulating Hormone values from cord blood in neonates

Yalemtsehay Mekonnen[1], Gizachew W. Hawariat[2], Bekele Chamiso[3], Friedhelm Raue[4]

Abstract

Objectives: To determine thyroid stimulating hormone (TSH) levels from cord blood in neonates and to establish the practice for possible application of congenital hypothyroidism screening in Ethiopia.

Methods: TSH was measured from cord blood of 1207 consecutive new-borns in the maternal wards of St. Paul, Ghandi Memorial and Tikur Anbesa hospitals in Addis Ababa, Ethiopia. TSH Immunoradiometric assay (IRMA) was used with anti-TSH coated beads and I^{125} labelled TSH monoclonal antibodies.

Results: Males (89.5%) and females (91.8%) had non detectable and/or less than 10 mIU/l TSH values. From the total of 548 male and 659 female neonates 10.2% males and 8% females had TSH values between 10-20 mIU/l. Only three neonates had raised TSH values, which was greater than 20 mIU/l.

Conclusions: Cord blood is practical and apparently simple to collect. It is applicable for blood spot analysis of congenital defects. It can be put in practice for large scale screening programmes such as congenital hypothyroidism (CH) in the Ethiopia situation where hospital discharges are within 24 hrs of delivery given all the necessary infrastructures in place. [*Ethiop.J.Health Dev.* 2003;17(2):125-130]

Introduction

Primary hypothyroidism is caused by a decrease in free thyroxin (T4) and free tiriodothyronine (T3) levels that trigger a rise in thyroid stimulating hormone (TSH) release from the anterior pituitary gland (1). A more severe condition is congenital hypothyroidism (CH), which results in defective brain development and disorganisation of interneuronal interactions (1,2). The aetiology of CH includes thyroid dysgenesis, thyroid dyshormonogenesis and hypothalamic-pituitary abnormalities. Transient hypothyroidism could also be induced due to iodine deficiency, drugs or maternal antibody productions (2,3). Iodine deficiency disorder (IDD) is a serious global health problem estimated to affect about one billion people across the world (4). The earliest sign of IDD is goitre. The disorders also include cretinism, neonatal hypothyroidism and congenital defects, as well as retardation of mental and physical development (5). Ethiopia belongs to those regions of the world where moderate to severe IDD prevails. The kinds of IDD in Ethiopia including goitre prevalence and cretinism have been reported (6,7).

An elevated TSH level in neonates usually requires an immediate thyroid hormone therapy. TSH measurement is essential to timely identify hypothyroid neonates in iodine deficient areas.

Since the 1970's, neonatal screening programs for hypothyroidism have been developed and have become popular worldwide. Specimens

[1]Department of Biology, Faculty of Science, P.O.Box 1176, email: yalemtsehay@yahoo.com; [2]Department of Internal Medicine; [3]Department of Paediatrics & Gynaecology, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia; [4]Endocrine Practice, 69120 Heidelberg, Germany

from cord serum and filter paper spotted with blood are utilised to measure TSH for CH screening (8,9). Due to early discharges from hospitals in Ethiopia, it is usually difficult to take blood samples from neonates after a few days of life. In the present work, results of cord blood sample TSH measurements are reported for an efficient CH screening.

Subjects and Methods

The subjects were 1207 neonates from three state-hospitals in Addis Ababa, Ethiopia. Samples were collected based on the consent of the mothers of the neonates who were born in the maternity wards of the respective hospitals from June to August 1999. The mother of every neonate born was asked to participate. The sample size was not predetermined it was based on the cord blood samples obtained during the screening period. Ethical approval was obtained from the concerned institution before collecting samples.

Neonatal data such as body weight, height, chest circumference and gestational age were collected. The latter was determined by counting weeks of pregnancy from the first day of the last menstrual period (LMP).

Umbilical cord blood collection

Umbilical cord blood spots were made on standardised filter paper (Guthrie card No. 2992, Scleicher & Schuell, Dassel, Germany) immediately after birth of the babies. Both sides of each spot on the Guthrie cards were fully saturated with the cord blood. The spots were dried at room temperature and cards were sealed and kept in a deep freezer until assayed in the laboratory of the Nuclear Medicine Unit of the Addis Ababa University. A questionnaire that registered neonatal features and the health history of the mothers was applied. Written consent was obtained from each mother before taking the cord blood samples.

TSH assay

The NETRIA TSH IRMA (NETRIA, the Royal Hospitals NHS Trust, St. Bartholomew's Hospital, London) used was an assay with anti-TSH coated beads and I^{125} labelled TSH monoclonal antibodies. The kit contained the necessary reagents and buffers. Three levels of quality controls namely low, medium and high were included. Polystyrene tubes (12x75mm) were labelled in duplicates for standards, quality controls and dried filter paper blood samples. Two filter paper spots measuring 6 mm in diameter each were punched from standards, quality controls and cord blood samples, and placed into their respective tubes. The assay was performed in accordance with the written instructions from the manufacturers.

Each tube containing 300 µl of I^{125} anti-TSH monoclonal antibodies were vortex-mixed except the total tubes. Each tube received one anti-TSH coated bead, and was kept rotating overnight at room temperature. All tubes were washed and aspirated with 2 ml wash buffer three times to remove unbound tracer. The paper discs were removed from each tube, and discarded during the first wash. Then the tubes were counted for 100 seconds in RIASTAR 5410 gamma counter to determine the amount of bound radioactivity in each specimen. A standard curve was generated using the software supplied with the gamma counter. Four parametric logistic curve-fitting procedures were used and the cord blood samples and the quality control results were read from the standard curve generated.

Three quality control pools were included right after the standards, and at the end of the samples each time the TSH assay was run. Error analysis was performed on the three quality control pools, and the average drift was not significant in all the tests. Within assay, variation was not significant for all the three pools but data were insufficient to check between batch trends. All the tests and data processing were done by one of the authors.

Statistical analysis

The data were analysed using Epidemiological Information (EPI-INFO) Version, 6.04b 1997. Mean, median and standard deviation (S.D) of the cord blood TSH values of the neonates were

considered to report the result of the screening. Analysis of variance was performed to test differences between means and the level of significance was taken at $p<0.05$.

Results

The mean, median and standard deviation (SD) of the cord blood TSH of the male and female neonates are presented in Table 1; 73.4% of the male neonates had TSH less than 10 and 10.2% of them had values between 10 and 20 mIU/l. Only 0.3% had TSH values greater than 20 mIU/l. Similarly 61.3% of the female neonates had less than 10 mIU/l TSH values and 8% had values between 10 and 20 mIU/l and 0.2% had greater than 20 mIU/l. The non-detectable TSH values for the male and female neonates were 16.1% and 30.5% respectively. Figures 1 and 2 show cumulative frequencies of TSH values for the males and females respectively. The neonates with cord blood TSH values greater than 20 mIU/l were two males and one female. The values were 25.4 mIU/l, 39.4 mIU/l for the males and 27.2 mIU/l for the female neonate. However, there was no goitre observed. The neonatal features of the neonates with greater than 20 mIU/l did not show any abnormality. For instance the birth weights were on average 3200 g, chest circumference 32.66 cm, head circumference 35 cm and body height 48.7 cm. Gestational age for the male neonate with the highest TSH value was 41 weeks with normal delivery.

Table 1: **Cord blood Thyroid Stimulation Hormone (mIU/1) values of the neonates**

TSH		n (%)	Mean	Median	SD
<10	Male	402 (73.4)	2.71	1.94	2.42
	Female	404 (61.3)	2.34	1.45	2.26
10-20	Male	56 (10.2)	12.7	11.58	2.5
	Female	53 (8.0)	13.1	12.26	2.3

Table 2 shows the mean, median and SD of the TSH values of the neonates by gestation age in weeks and birth weights in grams. As is evident from the values in the Table and tested by analysis of variance, there were no significant differences among the categories of the gestation age and birth weight in the TSH values. The overall response rate was about 95%.

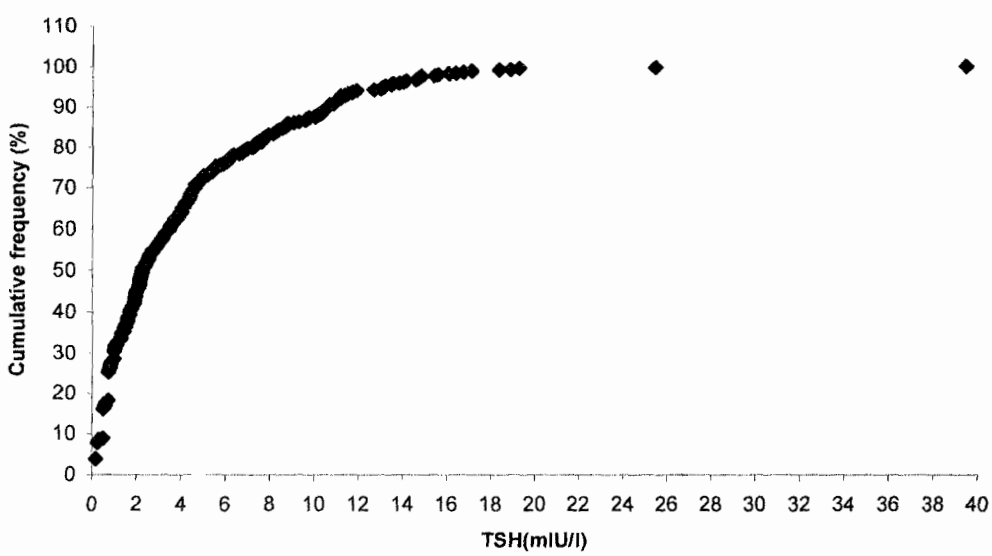

Figure 1: **Cumulative frequency of TSH values of the male neonates**

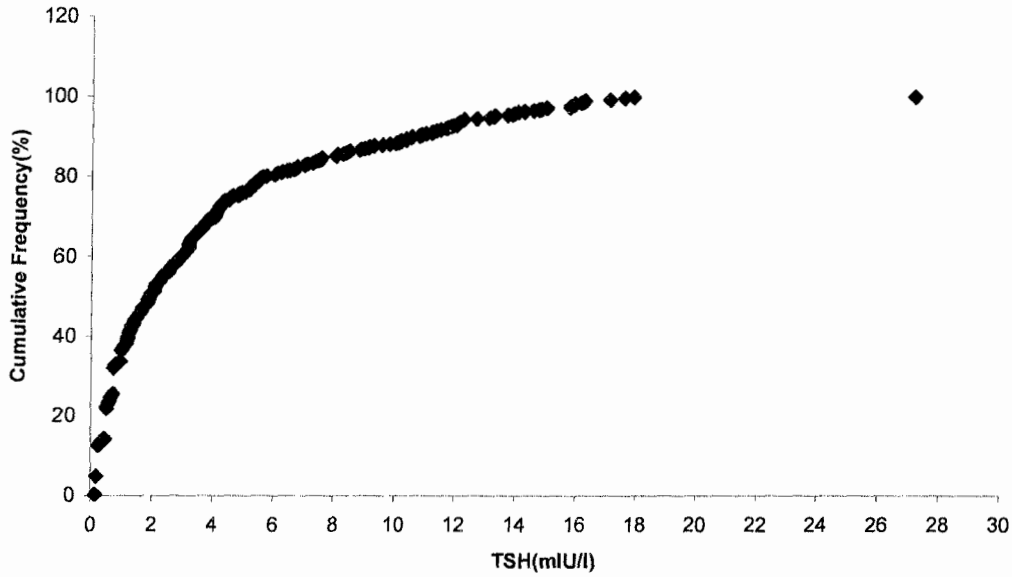

Figure 2: **Cumulative frequency of TSH values of the female neonates**

Table 2: **TSH (mIU/1) values of the neonates by gestation age and birth weight**

Gestation age (wk.)	Sex	N	Mean	Median	SD
<38	Male	71	3.54	2.14	3.69
	Female	64	3.98	2.32	4.39
38-42	Male	400	4.02	2.24	4.55
	Female	403	3.64	2.06	4.14
>42	Male	20	4.30	2.77	4.53
	Female	26	4.44	1.41	5.44
Birth weight (g)					
<250	Male	59	3.54	2.14	3.57
	Female	69	3.69	1.94	4.49
≥2500	Male	401	4.14	2.26	4.60
	Female	389	3.58	1.94	4.22

Discussion

The results demonstrated that there were three neonates with greater than 20 mIU/l TSH values in the study population. Some workers consider the threshold value for a significant TSH elevation to be 20-25 mIU/l for blood samples taken from heel pricks (2). Klein et al. (8) in North America showed that the TSH level in cord blood samples was less than 20 μU/ml in 85% and higher than 60 in 0.04% of the cases. In Ethiopia, Feleke and co-workers (10) measured TSH from heel pricks in neonates from six hours of life up to seven days old infants. They determined the cut off point for TSH to be 29.4 mIU/l. Considering the numerous iodine deficient areas in Ethiopia, it may be reasonable to take a lower cut off value of TSH. In the present work the 97.8th percentile of the TSH value was 15.4 mIU/l, which is closely comparable to the report of Kung et al (11); their 95th percentile using cord blood of neonates was 16 mIU/l. In areas of severe iodine deficiency and endemic goitre, the incidence of thyroid failure in the newborn can be as high as 1 in 10 (12,13).

The successful introduction of screening in the 1970's has enabled North America, Europe, to a limited extent Asia, Latin America and a few African countries to combat the ill effects of CH and saved lives (14,19). The frequency of CH in the earlier screenings was reported to be 1 in 6000 (20) in Canada, 1 in 5200 (15) and 1 in 4430 (21) births in North America. A screening program in South Africa has identified about 1 in 4143 hypothyroid incidence (22). These earlier screening schemes used cord serum, capillary heel-prick samples and a few used umbilical cord blood as well. In the 90's screening for CH has shown a lower incidence. In Spain, using umbilical cord specimens from neonates a frequency of 1:3400 was reported (23). Al-jurayyan and co-workers confirmed CH incidence of 1:3292 in Saudi Arabia (24).

Neonatal TSH has the major advantage of being the single indicator allowing prediction of possible impairment of mental development at a population level (12). With a properly collected specimen, primary TSH screening provides a cost-effective program with the highest detection rate and a very acceptable low recall rate (25).

The most discriminating screening test for primary hypothyroidism should be performed either on a cord specimen or a specimen collected after 24 to 48 hrs of life (2,9). The maternal wards of the hospitals in the present study give services to hundreds of women daily and the mothers are usually discharged in about 24 hrs. It is apparently difficult to get heel prick samples from new-borns at a later date. However, getting the cord blood on filter paper was found to be practical and the mothers consented without problem.

In conclusion, the results demonstrated that cord blood samples were easy to collect and applicable for blood spot assay. We emphasise that in the given situation in Ethiopia where hospital discharges are in about 24 hrs, cord blood samples for congenital screening should be put in practice given all other necessary infrastructures put in place.

Acknowledgements

The German Technical Co-operation (GTZ) supported this work. The authors would like to thank the mothers and the staff of the Maternity ward of the hospitals who collaborated in blood collection and Ato Negussu Worku for data analysis.

References

1. Foley TP JR, Malvaux P, Blizzard RM. "Thyroid Disease". In: Kappy MS., Blizzard RM, Migeon CJ, Eds. Wilkins The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence. Charles C. Thomas, 4th ed., Springfield, 1994; 457-533.
2. Fisher DA. Clinical Review 19. Management of congenital hypothyroidism. J Clin Endocrinol Metab. 1991;72:523-29.
3. Greuters A. Congenital hypothyroidism. Pediatr Annals 1992;21:15.
4. Hetzel BS, Potter BJ, Dulberg EN. The iodine deficiency disorders: nature, pathogenesis and epidemiology. In Bourne, GH, ed. Aspects of some Vitamins, Mineral and Enzymes in Health and Disease. World Review of Nutrition and Dietetics, Vol. 6. Karger, Basel; 1990;59-119.
5. Lamberg BA. Endemic goitre: idodine deficiency disorders. Ann Med. 1991;23:367-72.
6. Wolde-Gebriel Z, Demeke T, West CE, Van Der Haar F. Goitre in Ethiopia. British Med Journal 1993;69:257-68.
7. Neka-Tebeb H, Aboye C, Gebre P, Hailegiorgis B. Patterns of goitre in Sidamo awraja in Ethiopia and its relationship to piped water supply. East Afr Med J 1993;70:33-37.
8. Klein AH, Agustin AV, Foley TP. Successful laboratory screening for congenital hypothyroidism. Lancet 1974;2: 77-9.
9. Foley TP, JR,. Murphy WH. Primary thyrotropin screening for congenital hypothyroidism at birth, during the first

week of life and around one month of age comparison to primary T4 with secondary TSH screening. In: Burrow, GN, Dussault, JH, eds. Neonatal Thyroid Screening. Raven Press, New York, 1980;75-86.

10. Feleke Y, Enquosellassie F, Deneke F, et al. Neonatal congenital hypothyroidism screening in Addis Ababa, Ethiopia. East Afr Med J. 2000;77:377-81.
11. Limg AWC, Lao TT, Low LCK, Pang RWC, Robinson JD. Iodine insufficiency and neonatal hyperthyrotropineamia in Hong Kong. Clin Endocrinol 1997;46:315-19.
12. Delange F. Neonatal thyroid screening as a monitoring tool for the control of iodine deficiency. Acta Paediatr Suppl 1999;432: 21-4.
13. Kochupillai N. Neonatal hypothyroidism in India. Mt. Sinai J Med. 1992;59:111-15.
14. Delange F, Camus M, Winkler M, Dodion J, Ermans AM. Clinical aspects and systematic screening for congenital hypothyroidism in the newborn. Acta Paediatrica Belgica, 1977;suppl. VII, 30: 37.
15. Mitchell ML, Larsen PR, Levy HL, Bennett JE, Madoff MA. Screening for congenital hypothyroidism. JAMA 1978; 239:2348-51.
16. Fisher DA, Dussault JH, Foley TP et al. Screening for congenital hypothyroidism: Results of screening one million North American infants. J Pediatr 1979;94:700-05.
17. LaFranchi SH, Hanna CE, Krantz PL, Skeels MD, Miyahara RS, Sesser DE. Screening for congenital hypothyroidism with specimen collection at two-time period: results of the Northwest Regional Screening Program. Pediatrics 1985;76: 734-40.
18. Glorieux J, Dussault JH, Morissette J, Desjardins J, Letarte J, Guyda H. Follow-up at ages 5 and 7 years on mental development in children with hypothyroidism detected by Quebec screening program. J Pediatr 1986;107:913-15.
19. Fisher DA. Effectiveness of newborn screening program for congenital hypothyroidism: prevalence of missed cases. Ped Clin N Am 1987;34:881-90.
20. Dussault JH, Parlow A, Letarte J, Guyda H, Laberge C. TSH measurements from blood spots on filter paper. A confirmatory screening test for neonatal hypothyroidism. J Pediatr 1976;89:550-52.
21. LaFranchi SH, Murphey WH, Foley TP, Larsen PR, Buist NRM. Neonatal hypothyroidism detected by the Northwest regional screening program. Pediatr 1979; 63:180-91.
22. Bernstein RE, Hof J, Hitzeroth HW. Neonatal screening for congenital hypothyroidism. A decade's review, including South Africa. S Afr Med J. 1988;73:339-43.
23. Fernandez-Iglesias C, Florez IG, Rodriguez-Gonzalez MC, Gascon S. Neonatal screening for phenylketonuria and congenital hypothyroidism in Principado de Asturias (Spain) using two types of blood samples. Screening 1995;4: 131-38.
24. Al-Jurayyan AM, Al-Nuaim AA, El-Desouki MI, et al. Neonatal screening for congenital hypothyroidism in Saudi Arabia: results of screening the first 1 million new-borns. Screening 1996;4:213-20.
25. Van Vliet G, Merck AG. Thyroid Symposium. Neonatal Hypothyroidism: Treatment and Outcome. Thyroid 1999;9:79-83.